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Spreading depolarizations have prolonged direct current shifts and are associated with poor outcome in brain trauma

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Cortical spreading depolarizations occur spontaneously after ischaemic, haemorrhagic and traumatic brain injury. Their effects vary spatially and temporally as graded phenomena, from infarction to complete recovery, and are reflected in the duration of depolarization measured by the negative direct current shift of electrocorticographic recordings. In the focal ischaemic penumbra, peri-infarct depolarizations have prolonged direct current shifts and cause progressive recruitment of the penumbra into the core infarct. In traumatic brain injury, the effects of spreading depolarizations are unknown, although prolonged events have not been observed in animal models. To determine whether detrimental penumbral-type depolarizations occur in human brain trauma, we analysed electrocorticographic recordings obtained by subdural electrode-strip monitoring during intensive care. Of 53 patients studied, 10 exhibited spreading depolarizations in an electrophysiologic penumbra (i.e. isoelectric cortex with no spontaneous activity). All 10 patients (100%) with isoelectric spreading depolarizations had poor outcomes, defined as death, vegetative state, or severe disability at 6 months. In contrast, poor outcomes were observed in 60% of patients (12/20) who had spreading depolarizations with depression of spontaneous activity and only 26% of patients (6/23) who had no depolarizations (χ^2, $P < 0.001$). Spontaneous electrocorticographic activity and direct current shifts of depolarizations were further examined in nine patients. Direct current shift durations ($n = 295$) were distributed with a significant positive skew (range 0:51–16:19 min:s), evidencing a normally distributed group of short events and a sub-group of prolonged events. Prolonged direct current shifts were more commonly associated with isoelectric depolarizations (median 2 min 36 s), whereas shorter depolarizations occurred with depression of spontaneous activity (median 2 min 10 s; $P < 0.001$). In the latter group, direct current shift durations correlated with electrocorticographic depression periods, and were longer when preceded by periodic epileptiform discharges than by continuous delta (0.5–4.0 Hz) or higher frequency activity. Prolonged direct current shifts (>3 min) also occurred mainly

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within temporal clusters of events. Our results show for the first time that spreading depolarizations are associated with worse clinical outcome after traumatic brain injury. Furthermore, based on animal models of brain injury, the prolonged durations of depolarizations raise the possibility that these events may contribute to maturation of cortical lesions. Prolonged depolarizations, measured by negative direct current shifts, were associated with (i) isoelectricity or periodic epileptiform discharges; (ii) prolonged depression of spontaneous activity and (iii) occurrence in temporal clusters. Depolarizations with these characteristics are likely to reflect a worse prognosis.

Keywords: cortical spreading depression; electroencephalography; craniotomy; signal processing; acute brain injury

Introduction

Cortical spreading depolarizations describe a class of pathologic electrochemical waves that slowly propagate through cerebral grey matter at 2–5 mm/min. Imposing a significant metabolic challenge (Shinohara *et al.*, 1979; Turner *et al.*, 2007) and interrupting function in affected tissue, spreading depolarizations are increasingly recognized as an important pathophysiological mechanism in migraine, haemorrhagic and ischaemic stroke and traumatic brain injury (Lauritzen *et al.*, 2011). Following his initial discovery of these waves in the 'spreading depression of activity in the cerebral cortex' (Leão, 1944), Leão described their hallmark characteristic: a negative slow voltage variation lasting 1–2 min that he speculated was 'the expression of the depolarization of the normally polarized membrane of the neurons' (Leão 1947). Subsequent work confirmed that this negative shift of direct current potential reflects the mass depolarization of neurons and astrocytes that defines these events (Grafstein, 1956; Hansen and Zeuthen, 1981). The negative direct current shift closely relates to the breakdown of electrochemical gradients across cellular membranes and therefore generally mirrors the rise of $[K^+]_o$ and decline of $[Na^+]_o$ and $[Ca^{2+}]_o$ (Kraig and Nicholson, 1978; Hansen and Zeuthen, 1981).

Recovery from spreading depolarization depends on adequate cerebral blood flow and intact neurovascular coupling to match the increased demand for ATP and restore ion gradients by the Na^+/K^+ pump. Thus, following ischaemic stroke, depolarizations occur as graded events, with durations and effects that vary spatially from the core into normally perfused tissue (Nedergaard and Hansen, 1993; Koroleva and Bures, 1996; Dijkhuizen *et al.*, 1999; Nallet *et al.*, 1999). In the core, terminal depolarization develops and spreads multifocally, resulting in sustained tissue depolarization and persistent negative direct current shift. The wave then invades the poorly perfused penumbra as a peri-infarct depolarization, and additional peri-infarct depolarizations are subsequently triggered at the rim of the ischaemic core. Residual perfusion allows for recovery and restoration of membrane potentials in the penumbra, but the direct current shift durations are prolonged, lasting several minutes or more. These recurrent peri-infarct depolarizations cause progressive expansion of the infarct core (Back *et al.*, 1996; Busch *et al.*, 1996; Takano *et al.*, 1996; Shin *et al.*, 2006; Nakamura *et al.*, 2010). When the waves reach normally perfused cortex, they have a short, typical duration and do not directly cause cellular injury. Experiments in other models of ischaemia and subarachnoid haemorrhage also indicate that prolonged durations of direct current shifts distinguish depolarizations that promote lesion development (Dreier *et al.*, 1998, 2000; Oliveira-Ferreira *et al.*, 2010).

In contrast to ischaemic stroke, there is no evidence of prolonged depolarizations in animal models of traumatic brain injury (Sunami *et al.*, 1989; Rogatsky *et al.*, 2003; Williams *et al.*, 2005; von Baumgarten *et al.*, 2008). Depolarizations occur spontaneously and repetitively, but are of short duration, similar to those elicited in intact cortex that do not cause neuronal injury (Nedergaard and Hansen, 1988). Nonetheless, following traumatic brain injury in humans, repetitive depolarizations often recur in cerebral cortex with electrophysiological characteristics similar to the ischaemic penumbra (Astrup *et al.*, 1977, 1981). For instance, depolarizations may occur in cortex that is viable but isoelectric, i.e. with no high frequency (0.5–70 Hz) spontaneous activity (Fabricius *et al.*, 2006). Furthermore, in cortex with spontaneous activity, depolarizations may induce variable and sometimes prolonged depression of spontaneous activity, which may deteriorate with progressive loss of amplitude through a series of events (Hartings *et al.*, 2008).

These data suggest that, unlike experimental traumatic brain injury, depolarizations in human traumatic brain injury may have prolonged durations analogous to peri-infarct depolarizations in the ischaemic penumbra. Electrocorticographic studies of spreading depolarizations in human traumatic brain injury have been performed with alternating current-coupled bioamplifiers, which has precluded a direct assessment of the negative direct current shifts and, hence, the duration of cortical depolarizations (Strong *et al.*, 2002; Fabricius *et al.*, 2006; Hartings *et al.*, 2009*a*). However, we recently developed a signal processing method using an inverse filter to reverse the high-pass filter effects of alternating current-coupled amplifiers and recover full-band waveforms similar to direct current-coupled recordings (Hartings *et al.*, 2009*b*). In this study, we apply this technique to human electrocorticography to investigate the characteristics of negative direct current shifts of spreading depolarizations following moderate–severe traumatic brain injury in humans and their relationship to high frequency electrocorticographic activity.

Materials and methods

Clinical protocol

In the Co-Operative Study on Brain Injury Depolarizations, 53 patients with acute traumatic brain injury were prospectively enrolled at

four participating centres: King's College Hospital (London, UK), Virginia Commonwealth University (Richmond, VA, USA), University of Pittsburgh (Pittsburgh, PA, USA) and Mannheim University Clinic (Mannheim, Germany). Inclusion criteria were the clinical decision for craniotomy for lesion evacuation and/or decompression and age $\geqslant 18$ years. Patients with fixed, dilated pupils were excluded. Research protocols were approved by institutional review boards and surrogate informed consent was obtained for all patients. Research was conducted in accordance with the Declaration of Helsinki.

At the conclusion of surgery, an electrode strip was placed on the surface of the cortex for subsequent electrocorticographic recordings (Strong *et al.*, 2002; Fabricius *et al.*, 2006). The strip was placed near the injury focus on viable but often oedematous or contused cortex with a low load of subarachnoid blood. The lead wire of the strip was externalized through a burr hole in the skull (if the bone flap was replaced) and tunnelled beneath the scalp to exit 2–3 cm from the craniotomy margin. After surgery, patients were transferred to the intensive care unit where continuous electrocorticographic recordings were initiated. Throughout recordings, patients were ventilated and pharmacologically immobilized as required. Sedation was maintained with propofol or midazolam, and analgesia was provided with morphine or fentanyl. Phenytoin was administered for seizure prophylaxis according to local practice. Intracranial pressure was monitored, if clinically indicated, by a ventricular drainage catheter or an intraparenchymal intracranial pressure transducer (Codman). Intracranial pressure of >20 mmHg was typically treated by intravenous vecuronium, ventricular drainage, mannitol, hypertonic saline, mild hyperventilation and in a few patients by systemic cooling. The target level for cerebral perfusion pressure of >60 mmHg was achieved by intracranial pressure control, intravenous fluids and vasopressors. Electrocorticographic recordings were terminated and electrode strips were removed at the bedside by gentle traction when invasive neuromonitoring was no longer clinically required or after a maximum of 5 days. There were no haemorrhagic or infectious complications associated with the electrode strip. Clinical outcome was assessed at 6 months according to the extended Glasgow Outcome Scale and dichotomized to good (moderate disability or good recovery; 5–8) or poor (dead, vegetative state or severe disability; 1–4).

Electrocorticographic recordings

Electrocorticographic recordings were made from a linear subdural strip that consisted of six or eight electrodes, with 10 mm spacing between electrodes and 4.2 mm^2 of exposed platinum per contact (Wyler, Ad-Tech Medical). Electrodes were connected in a sequential bipolar fashion to alternating current-coupled amplifiers (Dual Bioamp or Octal amplifiers, ADInstruments), yielding five or seven recording channels (i.e. Ch.α = 1–2, Ch.β = 2–3, Ch.γ = 3–4, etc.). Ground was provided by an Ag/AgCl ground plate electrode placed on the patient's shoulder. Data were recorded and reviewed with 200 Hz sampling by a Powerlab 16/SP analogue-to-digital converter and LabChart software (ADInstruments). Amplifiers were set to a high-pass cut-off of 0.02 Hz to enable detection of slow potential changes resulting from negative direct current shifts.

Electrocorticographic recordings were first analysed for the occurrence of spreading depolarizations according to methods established by Fabricius *et al.* (2006). Briefly, depolarizations were identified by (i) the simultaneous occurrence of a slow potential change and depression of high frequency activity (0.5–100 Hz) in individual channels and (ii) the sequential occurrence of slow potential changes and depressions on adjacent channels that evidenced the spread of depolarization across the cortex. Slow potential changes also occurred in some cases when the high-frequency electrocorticography band was flat, or isoelectric, and therefore lacked depression periods. Slow potential changes occurring in a stereotyped and propagating manner in isoelectric tissue were termed isoelectric spreading depolarizations.

Processing of electrocorticography

As previously described, the negative direct current shifts of spreading depolarizations are reflected as multiphasic slow potential changes in these alternating current-coupled recordings (Fabricius *et al.*, 2006; Hartings *et al.*, 2006). Although alternating current-slow potential changes are useful for identification of spreading depolarizations, direct interpretation of their waveforms is precluded by two factors. First, alternating current-slow potential changes reflect distortions of the negative direct current shift of spreading depolarizations because of amplitude attenuation and phase shifts introduced by the high-pass filter stage of the amplifier. Second, slow potential changes in bipolar recordings may reflect the superposition (subtraction) of these occurring individually at each of the two electrodes connected in a recording channel (Hartings *et al.*, 2009*b*). Slow potential changes occurring on two electrodes will summate differently in a bipolar recording depending on their phase relationship and amplitudes.

Therefore, several *post hoc* signal-processing steps were used to overcome these obstacles and convert the bipolar alternating current-coupled recordings to full-band recordings that could be interpreted in a manner analogous to referential direct current-coupled recordings (Supplementary Fig. 1). First, the problem of superposition of slow potential changes occurring at both electrodes in a bipolar channel was overcome by computing derived pseudo-referential channels. The occurrence of depolarization at a particular electrode can be identified by the simultaneous occurrence of slow potential changes with phase reversals on adjacent channels (Fabricius *et al.*, 2006). Conversely, the absence of phase reversals (or furthermore, the absence of slow potential changes) indicates lack of depolarization activity. By these considerations, an electrode with a stable baseline and absence of slow potential changes can be chosen as an appropriate pseudo-reference. A pseudo-referential channel can then be derived by summing adjacent channels appropriately so that the derived channel reflects the potential difference between an active electrode of interest (i.e. with depolarizations) and the silent pseudo reference. By remontaging in this manner, complex slow potential changes consisting of superpositions of potentials occurring on both electrodes of a channel can be deconstructed into stereotyped simple slow potential changes that reflect depolarizations at only a single electrode (compare Ch.B = 2–3 in Supplementary Fig. 1A to traces in Supplementary Fig. 1B). To verify that potentials of interest in the derived channel are in fact attributable to the active electrode and not distorted by activity at the pseudo-reference, the constancy of the waveforms can be checked when necessary by using other available electrodes as reference.

After derivation of pseudo-referential channels, the distortions introduced by the high-pass filter stage of the alternating current amplifier are corrected by offline digital signal processing using an inverse filtering procedure as described previously (Hartings *et al.*, 2009*b*). In brief, the inverse filter is based on characterization of the transfer function (i.e. filtering effects) of the alternating current amplifier and its implementation as a digital filter. The approximate reciprocal of this transfer function, the inverse filter, can then be used for off-line processing of signals recorded with the corresponding amplifier to reverse the signal conditioning effects of the amplifier. Thus, the signal resulting from online acquisition with an alternating current-coupled amplifier, followed by offline inverse filtering, closely approximates the recordings of the same events made with a direct current-coupled

amplifier. With this procedure, there is complete recovery of signals with frequency content down to 0.001 Hz (period: 16 min 40 s). Because inverse filtering is a distributive operation, it may be performed either before or after (as shown in Supplementary Fig. 1) computation of derived channels to obtain pseudo-referential direct current recordings (Supplementary Fig. 1C). The use of these techniques to disambiguate the original recordings is illustrated by further examples in Supplementary Fig. 2.

To implement these procedures, original recordings acquired with the sequential bipolar montage were exported from LabChart software into MATLAB 7.2 (The MathWorks) for inverse filtering. The computational steps and parameters used for inverse filtering are provided in detail in Hartings *et al.* (2009*b*). Both original alternating current-coupled recordings and the corresponding inverse-filtered signals were then imported back into LabChart for remontaging and subsequent analysis.

Data set and analysis

Of the 53 patients studied, 10 exhibited at least one isoelectric depolarization, with or without additional depolarizations with spontaneous high-frequency electrocorticography and depression periods (Fig. 1). The isoelectricity present at the onset of isoelectric depolarizations developed as a result of prior depolarizations and persistent depression periods in five patients (e.g. Fig. 1B), and isoelectricity with recurrent isoelectric depolarizations was present from the beginning of recordings in four patients. Isoelectricity developed independent of depolarizations in only one case. The recordings of these 10 patients were further analysed to investigate direct current potential shifts of both types of depolarization. For each patient, one bipolar channel was selected for analysis based on a balance of the following criteria: (i) occurrence of $\geqslant 1$ isoelectric depolarization; (ii) availability of a pseudo-reference electrode with no depolarizations during periods of interest; and (iii) recording quality, including baseline stability of inverse-filtered signals. One patient was excluded because depolarizations occurred in near-synchrony at all electrodes and no electrode was available as a pseudo-reference. In the remaining nine patients, all depolarizations in the selected channels were analysed, including both isoelectric depolarizations and those with high-frequency electrocorticography depressions (Table 1). High-frequency electrocorticography depression periods were assessed in the bipolar recording channel and thus reflected depolarization on either or both of the adjacent electrodes connected in that channel. Bipolar recordings from minimally spaced electrodes are optimal for assessment of the electrocorticography suppression periods of spreading depression (van Harreveld and Stamm, 1951) and this practice has been the standard in clinical studies (Dreier *et al.*, 2006; Fabricius *et al.*, 2006; Dohmen *et al.*, 2008). Depression durations were scored beginning at the initial decrease in the integral (60 s decay time constant) of the power of the high-frequency electrocorticography and ending at the start of the recovery phase as described previously (Dreier *et al.*, 2006).

The negative direct current shifts of depolarizations were analysed after the derivation of two pseudo-referential channels from each bipolar channel, followed by inverse filtering, as described above. Direct current shift durations were measured starting at the time of dip below the baseline direct current level and ending at the time of recovery to this level (Fig. 7A, Dijkhuizen *et al.*, 1999; Hartings *et al.*, 2009*b*). Amplitudes were measured from the baseline direct current level to the peak negativity. Apparent propagation velocities were calculated as the 10 mm separation between electrodes divided by the time interval between the direct current shift onset at adjacent electrodes. Apparent velocities reflect both the wave speed and the angle of the wavefront relative to the electrode strip, which can not be determined (see Strong *et al.*, 2002). Mann–Whitney U-tests were used to compare values from different sampled populations. Kolmogorov–Smirnov tests were used to determine whether frequency distributions

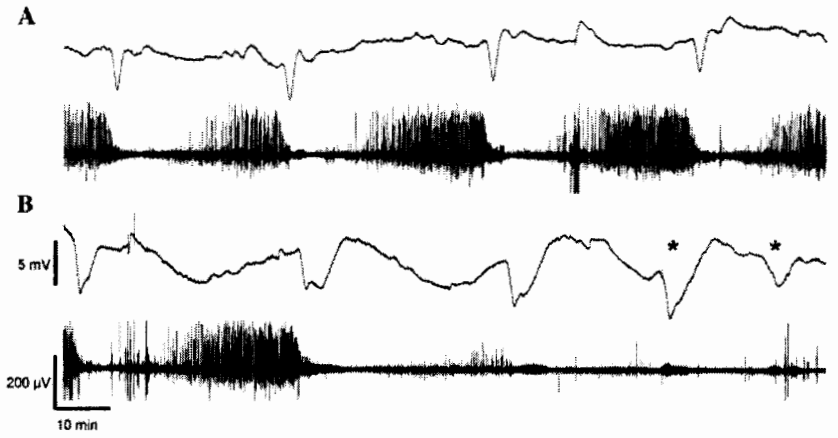

Figure 1 Prolongation of negative direct current shifts and development of isoelectricity in the high-frequency electrocorticogram. Recordings from Patient 2, a 47-year-old man who underwent decompressive craniectomy with non-evacuated mass lesion and recovered to a lower severe disability at 6 months. In 51 h of recording, 47 spreading depolarizations were observed, including two isoelectric depolarizations. (**A** and **B**) Two recording segments from a single channel beginning 22 h post-trauma. Each segment is 2 h 20 min in duration, with 8 h intervening between segments. 'Top' traces show full-band direct current potentials after inverse filtering; 'bottom' traces show high-frequency electrocorticography after high-pass filtering at 0.5 Hz. In (**A**), negative direct current shifts are 2.0–2.3 min and high-frequency electrocorticography depressions are 14–18 min. In (**B**), direct current shifts are more prolonged (4.2–6.7 min) and recovery of high-frequency electrocorticographic activity is delayed or absent. Asterisks denotes spreading depolarizations occurring while high-frequency electrocorticography remains depressed, or isoelectric, from prior events (isoelectric depolarization). Scale bars apply to (**A** and **B**).

Table 1 Summary of patients with isoelectric spreading depolarizations

Patient no.	Sex	Age	GCS motor score at admission	Recording duration (h)	[a]Direct current shift duration (min:s)		[b]HF-ECoG depression duration (h:min)		No. ISD	Six-month outcome
					Shortest	Longest	Shortest	Longest		
1	M	41	Untestable	68	1:36	5:03	0:07	3:10	9	Dead
2	M	47	Localizes pain	51	0:51	7:02	0:15	1:34	2	Lower severe disability
3	M	69	Untestable	87	1:23	4:48	0:14	0:38	10	Dead
4	M	70	No movements	100	1:13	9:15	0:12	1:44	26	Dead
5	F	79	Localizes pain	61	1:23	3:47	0:08	1:02	4	Dead
6	M	19	Abnormal flexion	127	1:08	2:33	0:03	1:02	2	Lower severe disability
7	M	54	Localizes pain	48	1:47	16:19	0:07	20:41	4	Lower severe disability
8	F	59	Localizes pain	11	1:06	8:01	No spontaneous HF-ECoG		17	Dead
9	M	39	Localizes pain	61	1:41	3:06	0:40	1:39	1	Upper severe disability
10	M	56	Normal flexion	72	Not analysed		Not analysed		7	Dead

Ten of 53 patients exhibited at least one isoelectric spreading depolarization (ISD). All depolarizations, including both isoelectric spreading depolarization and those with high frequency electrocorticography (HF-ECoG) depressions, were analysed in nine patients; lack of an appropriate pseudo-reference electrode precluded further analysis of the 10th patient.

a The shortest and longest duration negative direct current shifts observed on either of two electrodes in the bipolar recording channel.

b The shortest and longest periods of continuous high frequency electrocorticography depression induced by a single or multiple depolarizations.

GCS = Glasgow coma scale.

differed from normality and Kruskal–Wallis tests were used for non-parametric analysis of variance. Data are reported as median values (1st, 3rd quartiles).

Results

Patient data set and clinical outcome

Electrocorticographic recordings were performed during the period of intensive care [recording duration 71 h (48, 98)] in 53 patients who underwent craniotomies for treatment of acute traumatic brain injury. Thirty (57%) patients exhibited spreading depolarizations as identified by slow potential changes propagating along the electrocorticography electrode strip. In 20 (38%) patients, all depolarizations were preceded by spontaneous high-frequency electrocorticography, which was subsequently depressed. In the other 10 (19%), some depolarizations occurred when high-frequency electrocorticographic activity was flat, or isoelectric, and therefore lacked depression periods (Fig. 1; Fabricius *et al.*, 2006). All patients with these isoelectric spreading depolarizations had poor 6-month outcomes, defined as dead, vegetative state or severe disability (100%; 10/10; Table 1). In contrast, poor outcomes were observed in 60% of patients (12/20) who had spreading depolarizations with depression of spontaneous activity and in only 26% of patients (6/23) with no depolarizations (χ^2, $P < 0.001$).

Recordings from patients with isoelectric depolarizations were selected for analysis of direct current shifts (Table 1), since electrical silence defines the ischaemic penumbra (Astrup *et al.*, 1977, 1981) where prolonged direct current shifts occur in animal models. For each patient, one bipolar recording channel was processed off-line by remontaging and digital inverse filtering to yield two full-band (direct current) electrocorticography channels (i.e. pseudo-referential traces of activity from the two electrodes connected in the original bipolar channel) (Supplementary Fig. 1). In nine bipolar recording channels from nine patients, 170 spreading depolarizations were analysed, including 74 isoelectric depolarizations and 96 depolarizations with spontaneous high-frequency electrocorticography and depression periods. Since each depolarization wave usually propagated to both electrodes, a total of 295 direct current shifts were analysed. Direct current shift amplitude was 5.59 mV (4.17, 7.08) and propagation velocity was 3.53 mm/min (2.23, 5.84). There were no correlations of propagation velocity with direct current shift durations, high-frequency electrocorticography depression durations, or depolarizations type ($P > 0.05$).

Direct current shift durations

Direct current shift durations were 2 min 22 s (1:56, 3:04) but varied considerably within and between patients. Figure 1 shows representative traces of negative direct current shifts and associated high-frequency electrocorticographic activity. The distribution of all 295 direct current shift durations (Fig. 2A) differed significantly from both normal and lognormal distributions with a skew toward larger values (Kolmogorov–Smirnov tests, $P < 0.01$). We assumed that within this distribution there is a population of shorter duration events reflecting depolarizations as occur in healthy, normally perfused cortex, and that these conform to a normal distribution, whereas longer 'ischaemic' or 'peri-infarct' depolarizations (Nedergaard and Astrup, 1986; Nedergaard and Hansen, 1993; Back *et al.*, 1994; Nallet *et al.*, 1999) account for the positive skew. To distinguish these groups, tests for fit to a normal distribution were performed as the longest duration direct current shifts were removed from the distribution. At a cut-off of 3 min 30 s, the distribution no longer differs from normality (Kolmogorov–Smirnov tests, $P = 0.09$), and the best normal fit to the peak of the distribution is obtained at a cut-off of 3 min 0 s (Fig. 2B). For the best-fit distribution, the mean is 2 min 7 s

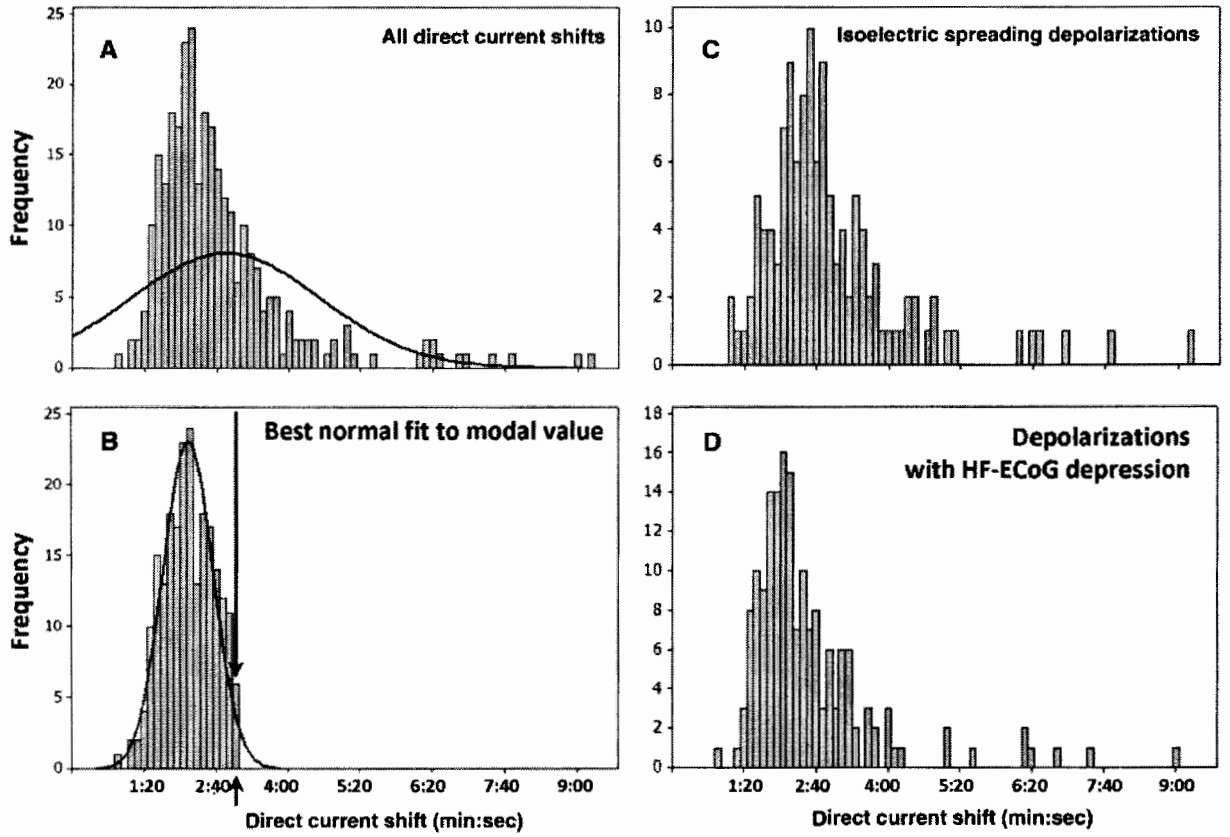

Figure 2 Evidence of prolonged direct current shifts. (**A**) Frequency histogram of direct current shift durations for 295 spreading depolarizations. With a positive skew, the distribution differs significantly from normality (solid line). Three longer direct current shifts up to 16 min 19 s are not shown. (**B**) The best normal fit to the peak was obtained by removing values >3 min. Arrows indicate two SD above the mean (3 min 1 s) and suggest a cut-off point between a normally distributed group of depolarizations and more prolonged events. (**C**) Direct current shift durations for isoelectric spreading depolarizations (ISD), i.e. those occurring during isoelectric high-frequency electrocorticography (HF-ECoG). (**D**) Direct current shift durations of spreading depolarizations that induced depression of baseline spontaneous high-frequency electrocorticography activity.

[standard deviation (SD) 0:27], indicating that 95% of short depolarizations are <3 min 1 s (mean + 2 SD) in duration. By this criterion, 75 (25%) of all 295 direct current shifts measured were of long duration (>3 min); these occurred in eight of nine patients.

Figure 2C and D compares the negative direct current shift durations of isoelectric depolarizations ($n = 127$) and depolarizations that depressed spontaneous high-frequency electrocorticographic activity ($n = 168$). Direct current shifts of isoelectric depolarizations were significantly longer [2 min 36 s (2:07, 3:24) versus 2 min 10 s (1:50, 2:47); Mann–Whitney U-tests, $P < 0.001$]. For instance, isoelectric depolarizations had long durations more frequently [33% (42/127) > 3 min compared to 20% (33/168) for those with depression periods], whereas depolarizations with depression periods were more frequently of very short duration [40% (67/168) ⩽ 2 min compared to 18% (23/127) for isoelectric depolarizations]. It is noteworthy, however, that there is substantial overlap in the two distributions. Therefore, isoelectric depolarizations are generally but not exclusively associated with longer duration direct current shifts.

Dependence of direct current shift and depression durations on baseline patterns

Spontaneous activity patterns are not uniform but rather vary widely after traumatic brain injury along a pathology continuum from normal to isoelectric. To determine whether direct current shift durations varied throughout this continuum, and not just between the presence/absence of spontaneous activity, the baseline high-frequency electrocorticography preceding each depolarization was classified into four categories: isoelectric, periodic epileptiform discharges only, delta activity only or mixed frequency content (Fig. 3A). Direct current shift durations differed significantly among the four patterns (Kruskal–Wallis, $P < 0.001$; Fig. 3B). Pairwise comparisons among categories revealed that direct

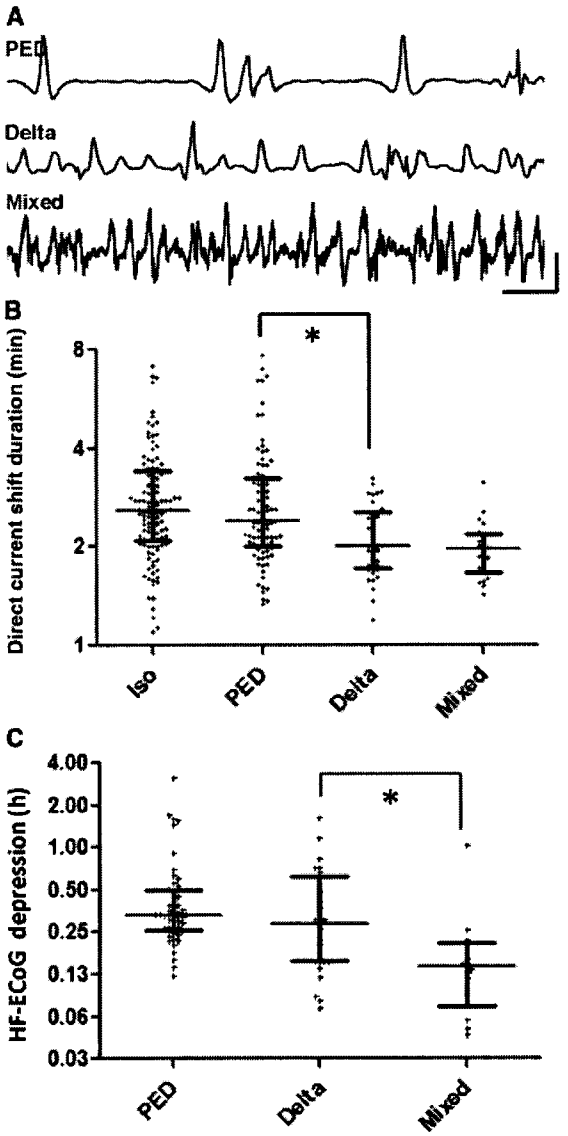

Figure 3 Association of baseline high-frequency electrocorticography patterns with depolarization characteristics. (**A**) Representative traces of baseline patterns: isoelectric (Iso; not shown); periodic epileptiform discharges (PED) only, without other continuous activity in any frequency band; continuous baseline activity in the delta (Delta; 0.5–4 Hz) range but not higher, with or without periodic epileptiform discharge superimposed; continuous baseline activity present at frequencies greater than delta (Mixed). Scale bars apply to all traces: 2 s, 200 μV. Direct current shift (**B**) and high-frequency electrocorticography (HF-ECoG) depression (**C**) durations are compared relative to the type of baseline activity occurring in the channel prior to the depolarization. Medians and inter-quartiles ranges are shown. In (**B**), eight data points >8 min for isoelectricity and periodic epileptiform discharge are not shown. Asterisks indicate significant differences.

current shift durations during isoelectricity and periodic epileptiform discharge spontaneous activity did not differ from each other, but were significantly longer than direct current shifts during delta and mixed-frequency activity (Mann–Whitney U-test, $P < 0.01$). Notably, the longest duration direct current shifts occurred only during periodic epileptiform discharges or isoelectricity. Similarly, high-frequency electrocorticography depression periods differed significantly among the baseline patterns of periodic epileptiform discharge, delta and mixed frequencies (Kruskal–Wallis, $P < 0.001$; Fig. 3C). In pairwise comparisons, depressions of mixed frequency content were shorter than those of periodic epileptiform discharge and delta activity (Mann–Whitney U-test, $P < 0.02$).

Correlation of direct current shift and depression durations

In clinical studies, the durations of high-frequency electrocorticography depression periods serve as a metric for the severity of depolarization activity, with prolongation of depression periods as an indicator of progressive metabolic deterioration (Dreier *et al.*, 2006; Dohmen *et al.*, 2008; Nakamura *et al.*, 2010). In contrast, the duration of depolarization, measured by the negative direct current shift, has been a standard metric in animal studies (Nedergaard and Astrup, 1986; Mies, 1997; Dijkhuizen *et al.*, 1999; Nallet *et al.*, 1999; Dreier *et al.*, 2001). To examine the relationship of these measures, high-frequency electrocorticography depression periods from bipolar recordings were compared to the direct current shift durations at each of the electrodes in the pair (Fig. 4). When high-frequency electrocorticographic activity was present in the baseline recording ($n = 89$), the subsequent depression periods during depolarizations significantly correlated with the maximum direct current shift duration among the two electrodes ($R^2 = 0.54$, $P < 0.001$). The slope of the linear regression line is 8.0, indicating that each additional minute of depolarization results in 8 min of further high-frequency electrocorticography depression.

Inter-depolarization intervals

Depolarizations often occur in temporal clusters as several or many events recurring at short intervals. Such clusters are a common pattern of ischaemic depolarizations with impaired neurovascular coupling (Shin *et al.*, 2006; Strong *et al.*, 2007), and as such, may be associated with prolonged direct current shifts. We therefore examined the association between inter-depolarization intervals and direct current shift durations. Figure 5A shows a histogram of time intervals since the prior depolarization for a total of 271 events. The distribution significantly differs from normality (Kolmogorov–Smirnov tests, $P < 0.001$), with the majority occurring at <1 h (209/271; 77%). Figure 5B shows the relationship between inter-depolarization intervals and direct current shift durations. Long duration direct current shifts occurred almost exclusively at short intervals: only two (3%) of 73 depolarizations with durations >3 min occurred at >2 h after the previous depolarization. In contrast, short direct current shifts occurred more frequently after long inter-depolarization intervals (41/198; 21%;

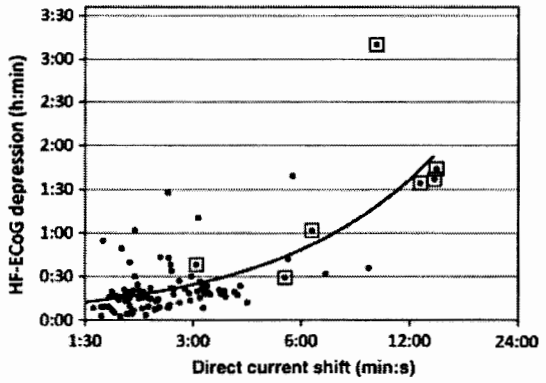

Figure 4 Correlation of direct current shift and high-frequency electrocorticography depression durations. High-frequency electrocorticography (HF-ECoG) depression periods from the bipolar recording channel versus the maximal direct current shift duration at either electrode. Square symbols show seven events in which continuous depression was due to a sequence of multiple depolarizations (two or three); in these cases, the direct current shift durations were summed. One event was excluded: a series of five depolarizations with total direct current shift duration of 49 min and depression duration of 20 h 41 min. Linear regression line is shown and the abscissa is plotted on a logarithmic scale.

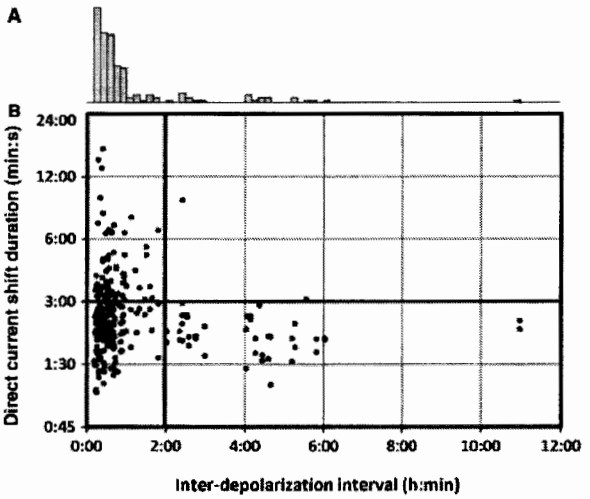

Figure 5 Inter-depolarization intervals. (**A**) Frequency histogram of inter-depolarization intervals with 10 min bins. The modal bin is 10–20 min. (**B**) Scatterplot of direct current shift duration versus the interval since the previous depolarization. Ordinate is plotted on a logarithmic scale.

χ^2, $P < 0.001$). These results suggest that long direct current shifts occur almost exclusively in clusters and that sparse depolarizations, with long intervals and short direct current shifts, constitute a more benign pattern than clusters.

Subtypes of direct current shift waveforms

The majority of direct current shifts had a typical morphology that consisted of a progressive negative direct current shift (time to peak negativity: 1 min 4 s; 0:49, 1:17), rounded trough and equal duration recovery followed by an overshoot. The total duration was 2 min 15 s (1:50, 2:49). However, two morphological subtypes with deviation from this pattern were observed on visual inspection (Fig. 6). The first subtype was an inverted saddle-shaped depolarization that had a slight rise after the initial negative peak, followed by a decline to a second peak negativity. In six different patients, 20 saddle-shaped depolarizations were visually identified and quantified. These direct current shifts had significantly longer total durations (4 min 36 s; 3:27, 6:48; Mann–Whitney U-test, $P < 0.001$) but similar rates of decline to the initial negative peak (1 min 0 s; 0:48, 1:14) compared with typical direct current shifts. In accordance with data in Fig. 3, saddle-shaped depolarizations occurred with isoelectricity in the high-frequency electrocorticogram ($n = 14$) or during periodic epileptiform discharges ($n = 6$).

The second waveform subtype was characterized by two stages in negative direct current shift development: an initial stage with shallow slope and a second stage with steeper slope. Thirty-five instances of these knee-shaped depolarizations occurred in five patients. Compared with typical direct current shifts, knee-shaped events had significantly longer declines to peak negativity (1 min 38 s; 1:13, 2:14) and slightly longer overall durations (2 min 35 s; 2:08, 3:43) (Mann–Whitney U, $P < 0.001$). Knee-shaped depolarizations were associated with all types of baseline high-frequency electrocorticographic activity: 12 with isoelectricity, 11 with periodic epileptiform discharges, one with delta and eight with mixed.

Figure 6B shows a scatterplot of the initial and recovery phase durations for all 289 direct current shifts. For 164 (57%) events, the recovery phase was longer than the initial phase. In this view, the saddle- and knee-shaped populations are distinguished with little overlap. All saddle-shaped events had longer recovery than initial phases while 29/35 (83%) knee-shaped events had longer initial phases. Of the seven electrodes where knee-shaped depolarizations occurred, three also recorded separate saddle-shaped events. Only one depolarization had both a knee-shaped declining phase and a saddle-shaped trough.

Discussion

Since the first use of subdural electrode strips to monitor patients with acute brain injury, it has become increasingly evident that, as in animals, at least some subset of spreading depolarizations contributes to secondary degenerative processes. Direct evidence is provided by serial imaging (Dreier *et al.*, 2006; Dohmen *et al.*, 2008) and monitoring of cerebral blood flow and oxygenation (Dreier *et al.*, 2009; Bosche *et al.*, 2010). Other evidence is provided by the persistent depression periods of high frequency cortical activity, which are easily measured using standard techniques.

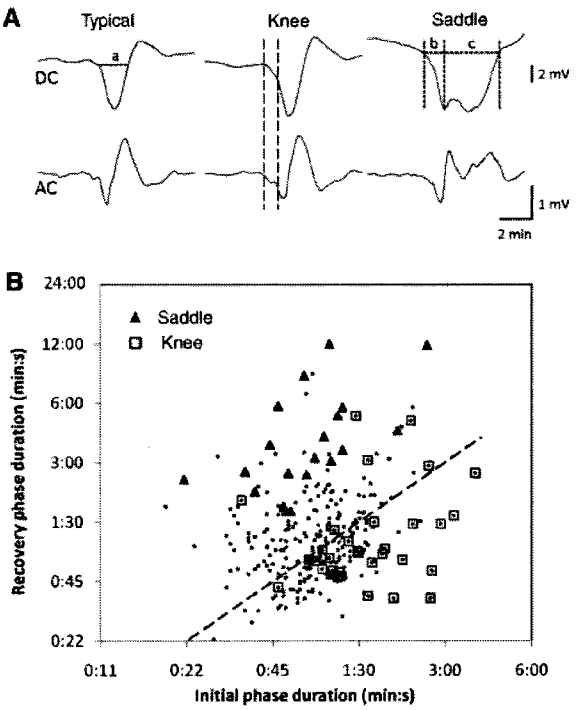

Figure 6 Subtypes of direct current shift waveforms. (**A**) Representative examples of typical, knee-shaped and saddle-shaped direct current shifts. Bottom row shows the alternating current-coupled recordings (0.02 Hz high-pass) and top row shows the direct current waveform after inverse filtering. Labelled dashed lines show the measurement of negative direct current shift duration (**a**), time to peak negativity (initial phase duration, **b**), and recovery phase duration (**c**). Note the two stages and inflection point (second vertical line) in the initial phase of the knee-shaped direct current trace, also observed in the alternating current trace. Negative is down. (**B**) Scatterplot of initial and recovery phase durations for all direct current shifts. Saddle- and knee-shaped events form separate clusters. Dashed line shows unity. Axes plotted on logarithmic scales.

However, direct measurement of the duration of cortical depolarizations—the gold standard in experimental studies—is more technically challenging and until recently has been lacking in humans (Dreier *et al.*, 2009; Oliveira-Ferreira *et al.*, 2010).

We developed an inverse filtering technique to allow assessment of the full-band direct current-electrocorticography potentials and here have analysed the direct current shifts of spreading depolarizations in relation to the high-frequency electrocorticography in a series of patients with traumatic brain injury. Our principal findings include: (i) the occurrence of spreading depolarizations, and particularly isoelectric depolarizations, is significantly associated with worse clinical outcome; (ii) a substantial subset (25%) of direct current shifts have prolonged durations, lasting up to 16 min; (iii) prolonged depolarizations occur more often, but not exclusively, in association with isoelectricity or periodic epileptiform discharges in the high-frequency electrocorticography; and (iv) the durations of negative direct current shifts correlate with high-frequency electrocorticography depression periods.

Prolonged direct current shifts in traumatic brain injury

Compared with ischaemic stroke and subarachnoid haemorrhage, there is a paucity of experimental literature on spreading depolarizations in acute traumatic brain injury. When depolarizations have been recorded in rodent traumatic brain injury models, they are typically of short duration and findings on their relationship to lesion growth are negative (von Baumgarten, 2008). Therefore, our findings from human clinical recordings are surprising and novel. Specifically, prolonged direct current shifts occurred in eight out of nine patients and accounted for 25% of all events analysed. Direct current shift durations exhibited a positive skew toward long events, consistent with the gradient of event durations and pathogenicities that occur in ischaemic stroke (Hossmann, 1996; Dijkhuizen *et al.*, 1999). Since prolonged direct current shifts can occur in association with high-frequency electrocorticography depressions (Fig. 2D), it is likely they also occurred in some of the 20 patients with high-frequency electrocorticography depressions only, who were not examined.

Since repolarization is extremely energetically demanding (Shinohara *et al.*, 1979), prolonged direct current shifts likely reflect poor tissue perfusion and/or impaired neurovascular coupling. Direct current shift durations are inversely related to the baseline level of cerebral blood flow (Mies, 1997) and are also dependent on the cerebral blood flow response: inverse neurovascular coupling causes transient hypoperfusion in the penumbra (Shin *et al.*, 2006; Strong *et al.*, 2007) and delays recovery from depolarization (Dreier *et al.*, 1998, 2000). However, frank ischaemia is not required for impaired neurovascular coupling and prolonged depolarizations. Rather, arterial hypotension is sufficient to abolish the normal hyperaemic response required for rapid repolarization, and depolarizations are therefore prolonged under hypotensive conditions (Sukhotinsky *et al.*, 2010). Rodent models of traumatic brain injury may lack prolonged direct current shifts because they fail to produce the degree of primary and secondary injury to cause such metabolic and cerebrovascular impairment as are associated, for instance, with mass lesions, intracranial pressure elevation and arterial hypotension in severe clinical traumatic brain injury. Indeed, hypotension is a risk factor for depolarizations in human traumatic brain injury (Hartings *et al.*, 2009a), and when cortical lesions in rats are compounded by secondary hypotension, prolonged direct current shifts are triggered in association with lesion growth (Trabold *et al.*, 2006).

Thus, prolonged depolarizations are observed in a wide variety of experimental preparations, including models of arterial occlusion (Back *et al.*, 1996; Busch *et al.*, 1996; Takano *et al.*, 1996), microvascular spasm (Oliveira-Ferreira *et al.*, 2010), erythrocyte toxicity (Dreier *et al.*, 1998, 2000) and cortical cold lesions with hypotension (Trabold *et al.*, 2006). Importantly, in every instance, prolonged direct current shifts are associated with lesion development. On the basis of this principle, we speculate that prolonged

depolarizations may also be a causative factor for secondary injury after human traumatic brain injury.

Direct current versus high-frequency electrocorticography

Cortical activity in the 0.5–70 Hz range is the focus of clinical neurophysiology practice and has been used in electrocorticography studies of acute brain injury to assess the functional impact of depolarizations. However, patterns of high-frequency electrocorticography depression are complex and their relationships to direct current shift durations have not been examined in detail. We found that when spontaneous activity is present, direct current shift and high-frequency electrocorticography depression durations are significantly correlated, even when depression periods are induced by multiple depolarizations (Dreier *et al.*, 2009). This result validates that high-frequency electrocorticography depression periods can be used as a surrogate marker of direct current shift durations, which are more difficult to assess. Although the depolarization block of action potential generation is a known factor causing high-frequency electrocorticography depression, other factors must be involved to account for the longer durations of the depression periods, which persist after repolarization. Depolarizations cause dendritic beading and loss of dendritic spines, which are reversible depending on the tissue's metabolic status and the number of depolarizations that occur (Risher *et al.*, 2010). This compromise of synaptic architecture may account for the delayed recovery of spontaneous activity, and also the progressive decrease in spontaneous activity amplitude induced by a series of depolarizations (Ohta *et al.*, 2001; Hartings *et al.*, 2006, 2008; Oliveira-Ferreira *et al.*, 2010).

We also found that direct current shift durations vary according to the pattern of baseline spontaneous activity. The longest depolarizations occur with periodic epileptiform discharges or isoelectricity in the baseline, and shorter depolarizations occur in the presence of continuous delta or higher frequency activity. Surprisingly, however, there was no sharp transition to longer durations associated with any one particular baseline pattern. This result supports the notion that spontaneous cortical activity patterns occur along a pathology continuum, thus reflecting the extent of injury and metabolic impairment as evidenced by the increasing durations required to repolarize cells and restore synaptic activity. Assessment of these baseline patterns may be useful for prognostication and patient monitoring. Although continuous EEG is currently used in acute brain injury for seizure monitoring, different patterns of non-seizure activity are largely ignored. However, there is growing interest in understanding what these other patterns represent (Hirsch *et al.*, 2005).

A continuum of spreading depolarizations

Based on experiments in focal cerebral ischaemia, Nedergaard and Hansen (1993) suggested that depolarizations can be divided into two categories described as short- and long-duration events. This concept has taken hold mainly because of the effect of long-duration peri-infarct depolarizations to enlarge infarctions, in contrast to classic spreading depressions occurring beyond the ischaemic zone (Strong and Dardis, 2005). Various duration thresholds have been proposed (Dijkhuizen *et al.*, 1999; Nallet *et al.*, 1999), and here we employed a cut-off of 3 min to distinguish long- and short-duration events as an *ad hoc* tool for describing the data. In previous studies without direct current recordings, we distinguished peri-infarct depolarization from cortical spreading depression based on the presence/absence of spontaneous activity and its depression (Fabricius *et al.*, 2006). However, it is important to emphasize that clinical data show no evidence for the existence of two populations of events with distinctly different pathologic relevance (Figs 2–4). Rather, the data represent a continuum that is also evidenced in the cerebral haemodynamic and oxygenation responses to depolarizations (Strong *et al.*, 2007; Dreier *et al.*, 2009; Bosche *et al.*, 2010). We therefore favour use of the term 'cortical spreading depolarization' to describe the whole spectrum of events, a neutral term without connotations regarding pathogenicity. Similarly, we have used the neutral term 'isoelectric spreading depolarization', rather than 'peri-infarct depolarization', to describe events in the electrophysiologic penumbra because they may not necessarily cause cortical lesions. In an ischaemia model using cortical application of the vasoconstrictor polypeptide endothelin-1, repetitive depolarizations occurred not only in a central region of isoelectricity that became infarcted, but also in an outer tissue belt of isoelectricity that survived. The critical difference between infarction and survival was prolonged direct current shifts in the central region (Oliveira-Ferreira *et al.*, 2010). However, even stereotyped depolarizations with short direct current shifts impose a substantial metabolic challenge (Hashemi *et al.*, 2009; Feuerstein *et al.*, 2010) and may cause secondary injury through other means (Gursoy-Ozdemir *et al.*, 2004).

Direct current shift morphologies

Two subtypes of depolarizations were identified based on the waveforms of the negative direct current shifts. Knee-shaped depolarizations had shallow and steep phases in the decline of the direct current potential whereas inverted saddle-shaped events had two negative peaks. Rarely were both features present in the same events, and quantification of durations evidenced separate populations with little overlap: saddle-shaped events had shorter initial phases but longer overall durations. On the other hand, subjective identification of these events was conservatively targeted toward extreme cases, and it is possible that events fall along a continuum between these ends of the spectrum.

Inverted saddle-shaped direct current shifts have been described frequently in microelectrode recordings of spreading depolarizations in animals. In hippocampal CA1, Herreras and Somjen (1993) found that depolarizations propagate in the apical dendritic layer with a long-duration, saddle-shaped morphology, but with a shorter, monophasic morphology in the somal layer. Since depolarizations can propagate through cortex independently at different depths, it was suggested that saddle-shaped morphologies might be observed when the larger dendritic voltages in superficial layers dominate the extracellular potentials. This was confirmed by

Richter and Lehmenkühler (1993). Thus, different direct current shift morphologies in our recordings may reflect propagation in deep versus superficial cortical layers that could arise from multiple sources of wave ignition. Differences in cytoarchitecture of recorded sites are an unlikely explanation because both knee- and saddle-shaped depolarizations were often recorded from the same electrode. Whether distinguishing these morphological subtypes has any implications for pathophysiology or therapy is unknown.

Electrocorticography methodology

One shortcoming of our methods is that longer direct current shift durations may have been underestimated due to the lower frequency limit of the inverse filtering method (0.001 Hz). In ischaemia models, recovery from an initial depolarization is often only partial and may progress over tens of minutes (Koroleva and Bures, 1996; Dijkhuizen *et al.*, 1999; Oliveira-Ferreira *et al.*, 2010). Such activity may extend below 0.001 Hz and furthermore is difficult to distinguish from a fluctuating baseline and direct current noise. Another limitation is imposed by use of internal references from the electrode strip. Direct current potentials from some electrodes could not be analysed because of the lack of an inactive channel to use as a pseudo-reference. Additionally, analysis of multiple channels is laborious since different electrodes or events often require different pseudo-references. Our initial experience suggests that direct current-coupled subdural recordings with an external reference are feasible, and given the importance of the direct current shift duration, may provide an attractive future alternative (Dreier *et al.*, 2009; Oliveira-Ferreira *et al.*, 2010).

Conclusion

We found that the occurrence of spreading depolarizations in the acute phase after clinical traumatic brain injury is significantly associated with worse clinical outcome. A simple electrocorticographic scoring method provided a graded prognosis corresponding to 100% (isoelectric depolarizations), 60% (depolarizations with depression periods) and 23% (no depolarizations) of poor outcomes. Analysis of depolarization durations, as measured by negative direct current shifts, provided further insight into this classification scheme. In particular, prolonged depolarizations were associated with (i) isoelectricity or periodic epileptiform discharges; (ii) prolonged depression of spontaneous activity; and (iii) occurrence in temporal clusters. Depolarizations with these characteristics are likely to reflect a worse prognosis. The independent prognostic value of electrocorticography monitoring needs to be evaluated in future multivariable analysis taking account of known risk factors for adverse outcomes.

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Supplementary material

Supplementary material is available at *Brain* online.

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